nsls
IR UV X

Science Highlights

from the National Synchrotron Light Source

BEAMLINE
X12B

PUBLICATION
Zhou, T. *et al.*, "Structure of Human Nicotinamide/Nicotinic Acid Mononucleotide Adenylyltransferase: Basis for the Dual Substrate Specificity and Activation of the Oncolytic Agent Tiazofurin," *J. Biol. Chem.*, **277**, 13148-13154 (2002).

FUNDING
The Robert A. Welch Foundation
National Institutes of Health

FOR MORE INFORMATION
Hong Zhang, Assistant Professor,
Department of Biochemistry, University of Texas Southwestern Medical Center,
Dallas, Texas
zhang@chop.swmed.edu

Structure of Human Nicotinamide/Nicotinic Acid Mononucleotide Adenylyltransferase (NMNAT)

T. Zhou[1], D. R. Tomchick[1], D.D. Binns[1]. N.V. Grishin[1], H. Zhang[1], O.V. Kurnasov[2], A.L. Osterman[2] and V.E. Marquez[3]

[1]Univ. of Texas Southwestern Medical Center at Dallas, [2]Integrated Genomics, Inc., [3]Frederick Cancer Research and Development Center, National Cancer Institute,

Nicotinamide adenine dinucleotide (NAD) is a coenzyme (nonprotein part of an enzyme) involved in many metabolic reactions inside the cell, as well as DNA repair and calcium signaling. NAD results from the addition of adenylate (one of RNA's building blocks) to the molecule nicotinamide mononucleotide (NMN), a process catalyzed by the enzyme NMN adenylyltransferase (NMNAT). The enzyme is also involved in activating the anticancer agent, tiazofurin. To understand better the role of NMNAT in NAD biosynthesis and tiazofurin conversion, scientists have used x-rays produced at the National Synchrotron Light Source and the Advanced Photon Source to determine the structure of NMNAT with different ligands, providing insight into the molecular mechanisms of the enzyme's active site.

Nicotinamide adenine dinucleotide (NAD) is a coenzyme (nonprotein part of an enzyme) that has been known for decades as the major hydrogen donor or acceptor in many metabolic reactions inside the cell, as well as the modification of nuclear proteins by ADP ribosylation, a process involved in DNA repair and the regulation of genomic instability.

Scientists have recently found that NAD is a substrate or a co-factor in the SIR2-like histone deacetylase, responsible for gene silencing and the increase of lifespan of many species, including yeast, worm, and mammals. Also, several derivatives of NAD are intracellular calcium mobilizing agents in various calcium signaling pathways.

NAD results from the addition of adenylate (one of RNA's building blocks) to the molecule nicotinamide mononucleotide (NMN), a process catalyzed by the enzyme NMN adenylyltransferase (NMNAT). NAD can also be synthesized by the following two successive processes: addition of adenylate to the molecule nicotinate mononucleotide (NaMN), leading to nicotinate adenine dinucleotide (NaAD), and addition of an amide group (organic compound containing the $CONH_2$ radical) to NaAD, leading to NAD. The first process is catalyzed by NMNAT and the second by NAD synthetase. (A synthetase is an enzyme that catalyzes the union of two molecules.) The processes are illustrated at: http://hhmi.swmed.edu/Labs/hz/nad1.htm

Human NMNAT also catalyzes part of the metabolic conversion of the anti-cancer agent tiazofurin, to its active form, tiazofurin adenine dinucleotide (TAD), an NAD analogue. The development of tiazofurin resistance has been shown to relate mainly to a decrease in NMNAT activity. The process is illustrated at: http://hhmi.swmed.edu/Labs/hz/tiazofurin.htm

Hong Zhang (lead author, right) and two members of her team: Subramanian Karthikeyan (seated) and Xuejun Zhang, at the University of Texas Southwestern Medical Center in Dallas.

Human NMNAT, located within the cell nucleus, recognizes both NMN and NaMN substrates. To understand the enzymatic properties of NMNAT and how its activity is regulated, we have solved the crystal structures of human NMNAT attached to several ligands, including NAD, NaAD, and TAD, to 2.2-angstrom (Å) resolution.

The data were produced and collected by using x-rays at beamline X12B of the

National Synchrotron Light Source and beamline 19ID of the Advanced Photon Source at Argonne National Laboratory.

Our results show that NMNAT bind to NMN and NaMN with high affinity. In particular, a water molecule in the active site appears to play a critical role in the recognition of NaMN (**Figure 1**).

By looking at the structure of NMNAT attached to TAD, we found that TAD molecule adopts a similar conformation as the native ligand NAD, and that TAD and NAD form essentially the same interactions with NMNAT. Additional functional groups on tiazofurin molecule also may enable more favorable interactions between tiazofurin and NMNAT. Further kinetic measurement will be needed to fully characterize the molecular interactions between human NMNAT and tiazofurin.

The results presented here represent a first step in our effort to decipher the metabolic pathways of NAD biosynthesis and how it is regulated in humans. We have recently identified an additional cytosolic form (inside the cell cytoplasm instead of the cell nucleus) of human NMNAT; its structural determination is well underway.

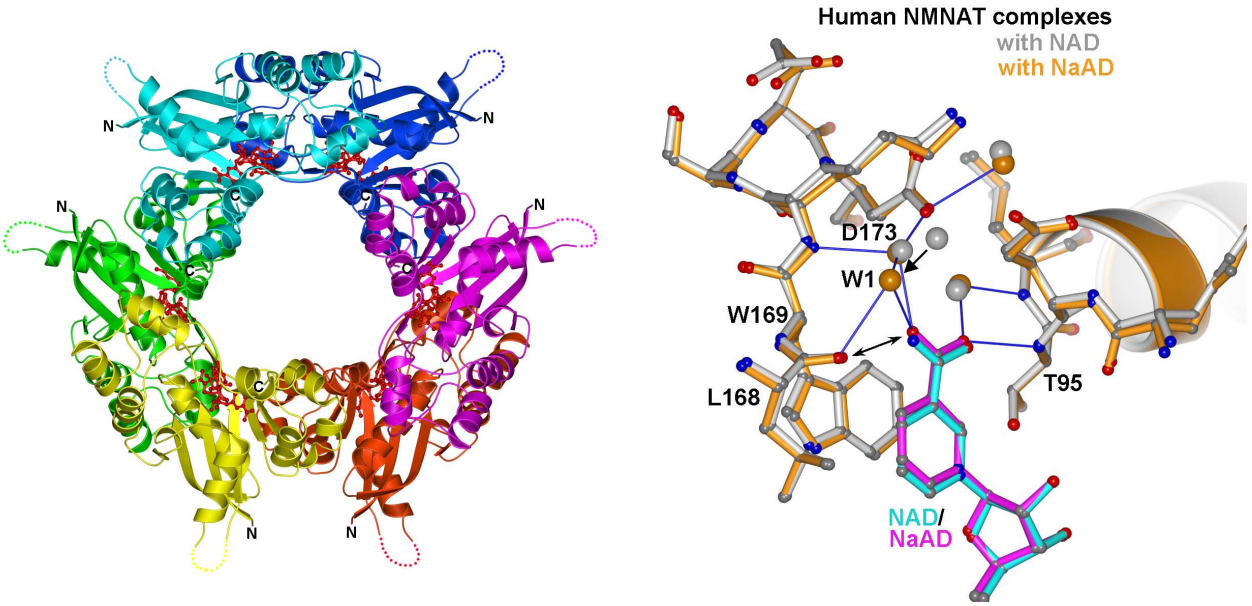

Figure 1. *Left: Ribbon diagram of human nicotinamide mononucleotide adenylyltransferase (NMNAT) hexamer.* Right: *The active site of human NMNAT with nicotinamide adenine dinucleotide (NAD) and nicotinate adenine dinucleotide (NaAD). A water molecule (denoted w1) in the active site of NMNAT changes location upon binding to different substrates.*